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Injection of innocuous oils to create reactive barriers for bioremediation: Laboratory studies

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Abstract

In situ groundwater remediation may be achieved using stationary permeable barriers created by the injection of a substrate, such as innocuous vegetable oil, into the contaminated aquifer. The oil provides the electron donor stimulating microorganisms to degrade or sequester many contaminants. At present, little is known about the best procedures to use when injecting oil into an aquifer. In this investigation, laboratory column and sand tank studies were used as model systems to explore the effect of different injection parameters on the distribution of oil emulsions into water-saturated sand. The parameters investigated included injection pressures of 70, 1400 and 18,000 KPa; injection times of 15, 30, 60 or 120 s; and the influence of an emulsifier, polyoxyethylenesorbitan monooleate (Tween 80), upon the distribution of the injected oil. The longest injection patterns were achieved at 18,000 KPa. A pattern that was 46 ± 1.8 cm long was produced with an 18,000 KPa injection for 60 s. Increasing the injection time to 120 s increased the length of the pattern by only 6.5%. Tween 80 at concentrations of 0.05% increased the width of the injection patterns but did not increase the length of the pattern. A multi-ported injection probe might be used to create in situ permeable barriers approximately 1 m wide. Published by Elsevier B.V.

Keywords: Biobarrier; Bioremediation; Denitrification; Nitrate; Perchlorate; Denitrifying wall; TCE

1. Introduction

For the remediation of groundwater contaminants, in situ treatment approaches that use stationary permeable barriers are a promising approach. Such systems are simpler and less

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expensive than pump-and-treat procedures (Streile et al., 1991; Green and Shelef, 1994). The stationary permeable barrier makes use of a portion of the aquifer as a primary reactor to cleanse contaminants from contaminated water. For biological treatment, the barrier may be established by the injection of an electron donor such as a soluble carbon substrate into a portion of the aquifer. In soils and groundwaters that are below the root zone, microbial activity is often restricted by the availability of a suitable substrate or electron donor (Myrold and Tiedje, 1985). The substrate stimulates microbial activity and the degradation of the contaminant. The barrier is placed beneath or downstream of the contaminant plume and the contaminant is removed as the natural movement of the water carries the contaminated water through the stationary barrier. A difficulty associated with using soluble substrates for long-term treatments is that the flowing groundwater will carry the substrate away from the injection site. Thus, with soluble substrates the continued functioning of the reactive barrier requires that a continuous or nearly continuous supply of nutrients be pumped into the aquifer to provide substrate to the contaminant-degrading microorganisms.

Remediation approaches based on the injection of soluble nutrients include the expense of nutrient reservoir tanks and pumps required to deliver the nutrients and the cost of continued monitoring of the injection process. In addition, the process may fail due to biofouling at or near the injection points (Hamon and Fustec, 1991; McMahon et al., 1998; Hunter, 2001a). A simpler in situ approach may involve the injection of a non-aqueous phase liquid (NAPL), such as vegetable oil, into the aquifer to provide an electron donor for the contaminant-degrading microorganisms. At the start of the remediation project, multiple injections would be needed to distribute the substrate and establish the barrier. The initial injection would need to provide sufficient substrate to allow the barrier to function for an extended period, perhaps years (Robertson and Anderson, 1999; Schipper and Vojvodic-Vukovic, 2001; Hunter, 2001b).

Vegetable oil is a candidate for use as a substrate in stationary barriers because it is nearly insoluble in water, is cheap, innocuous, has a high-energy content, and is readily degraded by microorganisms. Laboratory studies have shown that vegetable oil emulsions can be injected onto soil columns to form stationary permeable barriers (Hunter et al., 1997). The barriers, consisting of oil that is trapped on or between soil particles, remain stationary or nearly stationary as water is pumped through them. Such laboratory and pilot scale barriers have been effective at stimulating the microbial degradation of nitrate and perchlorate as water containing these oxyanions was pumped through oil containing permeable barriers (Hunter and Follett, 1995; Hunter et al., 1997; Hunter, 2002). Vegetable oils have also been used in laboratory reactors as a substrate to support the reductive dehalogenation of dichloroethene, trichloroethene, and perchloroethene (Lee et al., 2000; Zenker et al., 2000). However, little information is available on the best methods to use when injecting oil into an aquifer.

The most common method used to inject vegetable oil into aquifers for remediation purposes involves direct low-pressure injection of vegetable oil as a NAPL followed by injections of water to push the oil away from the injection site. This method has been used in field studies designed to remediate groundwater contaminated with chlorinated solvents (Wiedemeier et al., 2001; Lee et al., 2001; Waddill et al., 2002). Lee et al. (2001) has also proposed the use of an emulsifier and has compared direct injection of oil as an NAPL

with injection of an emulsified oil. They found that the emulsified oil mixture gave greater distribution of the oil and enhanced the remediation process. However, details describing the injection pressure, type of emulsifier used, or emulsifier to oil ratios were not presented. The present study used sand column and sand tank studies to investigate the effect of different injection parameters on the distribution of oil emulsions in sand.

2. Materials and methods

2.1. Column emulsifier studies

In a series of column studies this investigation evaluated the effect of polyoxyethylenesorbitan monooleate (Tween 80) on the distribution of an injected oil and water emulsion. Columns were horizontally mounted 2.6 by 60 cm glass tubes with a total volume of 300 mL. Water-saturated washed quartz sand, 0.35 mm sieve size, packed to a bulk density of 1.4 to 1.5 g cm^{-3}, was used as the support matrix. Column pore volume was 126 mL. A 50 mL emulsion containing soybean oil and water (1 : 3 v:v) was formed by mixing with a Polytron homogenizer operated at full speed for 3 min. The emulsions were injected into the inlet end of columns using a small gear pump operated at 100 KPa for ~12 s. Columns were filled with water before the injection and water displaced by the injection was allowed to escape from the opposite end of the column. A dye, Oil Red O, was included in the oil phase of the emulsion at 0.125% so that the distribution and movement of the oil in the emulsion could be visually monitored through the glass sides of the column as water was pumped through the columns. Tween 80 at 0.002%, 0.005%, 0.01%, 0.02%, 0.05%, 0.1%, and 0.2% was added to the aqueous phase before the emulsion was formed. The distribution of the oil and water emulsion was measured immediately after injection and again after 1.5 L of water was pumped through the columns at a flow rate of 1 L day^{-1}.

2.2. Column flow studies

This study used horizontally mounted 2.6 by 60 cm sand columns to confirm the ability of the oil and water emulsion to remain in place as a nearly stationary barrier while water was pumped through the column based barriers. The column based barriers were formed by injecting a 50 mL oil and water (1 : 3) emulsion onto the columns. The emulsion was formed with the aid of a Polytron homogenizer and injected with a gear pump as described above. For this study a single concentration of Tween 80, 0.02%, was used and added to the aqueous phase before the emulsion was formed as was done in the study above. The emulsions were injected into the inlet end of the sand columns using the gear pump described above. For this study 2 control columns and 4 treatment columns were used. Control columns were identical to treatment columns except that no water was pumped through the control columns, whereas with the treatment columns reconstituted water was pumped through them at a rate of ~1 L day^{-1} for 60 days. At the end of the study the amount of oil in the sand was determined via a combustion procedure outlined in Section 2.4.

2.3. Sand tank studies

A 61 × 61 × 152 cm (height : width : length) metal tank was filled with water-saturated sand. The sand used here was the same as was used in the sand column studies. A cover, formed from a heavily reinforced 54 by 125 cm sheet of plywood, was anchored in place on the top of the sand with six threaded metal rods, to serve as a pressure plate to prevent the upward movement of the sand during an injection. Injections of a 1 : 3 oil and water emulsion were made at 70, 1400 or 18,000 KPa for 15, 30, 60 or 120 s. Included were a series of studies where 0%, 0.02%, 0.05%, 0.1%, and 0.2% Tween 80 were incorporated into the aqueous phase. The point of injection was 30 cm from the bottom of the sand tank and 37 cm from the injection end of the tank. Perforated pipes, 1.9 cm in internal diameter, wrapped with a fiberglass screen having a 1 mm mesh, were placed at 60 cm intervals along the bottom and sides of the tank (Fig. 1). An oil soluble dye, 0.2% Oil Red O added to the oil phase, was included in the oil and water emulsion. The dye allowed us to visualize the distribution pattern of the oil in the sand. After each injection, the cover of the sand tank was removed, the tank drained, and the sand scraped off the surface in 5 cm layers. Sand samples were collected at 6 cm intervals along the length and width of the injection pattern and analyzed for oil content. The amount of oil in the sand was determined via the combustion procedure outlined in Section 2.5. The distribution of the Oil Red O stained soybean oil was recorded by digital photography. A marker of known area was placed within each photograph and, with the marker as a reference; the area over which the soybean oil was dispersed was estimated with the pixel counting option within the Adobe Photoshop v 5.02 photographic program (Adobe Systems Inc).

The injection emulsion was prepared in a 60 L tank connected to a 0.5 hp (373 W output) centrifugal circulation pump. The input end of the pump was connected by pipe to the bottom of the emulsion tank while the output end of the pump was connected to a return pipe placed near the top of the emulsifier tank. When the pump was turned on, liquid in the tank was pumped from the bottom of the tank, through the pump, and returned to the top of the tank. The pump was operated in this recirculation mode for

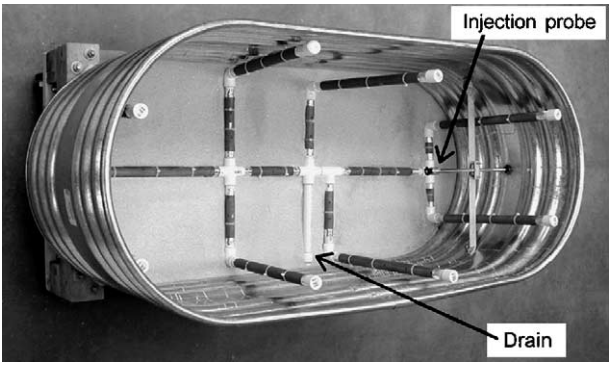

Fig. 1. Interior of sand tank showing the internal pipes, drain and injection point.

30 min before an injection. This procedure mixed and emulsified the liquids in the emulsion tank. A valve arrangement was used to divert the flow of the circulation pump to the input of a high-pressure injection pump. A high-pressure piston pump was used for the 1400 and 18,000 KPa injections. Pressure was controlled by a nozzle attached to the end of the injection probe. The centrifugal pump alone was used for the 70 KPa injection studies.

2.4. Determination of the amount of oil present in the sand columns by combustion

Sand samples from columns were collected at 2.5 cm intervals or segments for the first 30 cm of column length and 5 cm for the next 10 cm of column length, while the final 20 cm of the sand column made up the last segment. The sand samples were mixed and sub-sampled. The sub-sample, weighing approximately 10 g, was dried for 2 h at 105 °C to remove water, weighed, heated to 500 °C for 4 h, cooled, and weighted again. The decrease in the weight of the dried sand was used as a measure of the amount of oil in the sand.

2.5. Determination of the amount of oil present in the injection patterns by combustion

Sand samples from the sand tank were collected along a line, from the injection point outward, at 6 cm intervals. The sand samples were mixed and sub-sampled. The sub-sample, weighing approximately 10 g, was dried for 2 h at 105 °C to remove water, weighed, heated to 500 °C for 4 h, cooled, and weighted again. The decrease in the weight of the dried sand was used as a measure of the amount of oil in the sand.

2.6. Effect of Tween 80 on nitrate removal, microbial respiration, and denitrification

A microcosm study was conducted to determine if low concentrations of Tween 80 had an influence on several important microbial physiological markers. Microcosms were 118 mL serum bottles containing 25 mL of growth media under a He atmosphere. The growth media, adopted from Kuykendall (1987), had the following composition: 1.3 g Hepes (*N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid), 1.1 g MES [2(*N*-morpolino)ethanesulfonic acid], 0.0067g $FeCl_3 \cdot 6H_2O$, 0.18 g $MgSO_4 \cdot 7H_2O$, 0.013 g $CaCl_2 \cdot 7H_2O$, 0.25 g Na_2SO_4, 0.32 g NH_4Cl, 1.09 g $NaNO_3$, 0.92 g soybean oil, and 0.125 g Na_2HPO_4 per liter. Media pH was 6.9. Tween 80 at concentrations of 0%, 0.0125%, 0.025%, 0.05%, 0.1%, or 0.2% was added where indicated. Each Tween 80 treatment was replicated five times. A soil inoculum was also incorporated into the buffer (see below). Purging with helium displaced oxygen in the buffer. Deoxygenated buffer was added to the microcosm bottles and the bottles were capped with serum stoppers while in a helium filled glove bag. Microcosm bottles were removed from the glove bag and 2 mL of acetylene was added to each bottle to block the conversion of N_2O to N_2. Next, the microcosms were incubated in the dark at 100 rpm and 28 °C. At 0, 2, 4, 7, and 10 days incubation, 0.5 mL liquid and 5 mL gas samples were collected with syringes. Samples were analyzed for NO_3^- as described previously (Hunter et al., 1997) and for CO_2 and N_2O as described below.

2.7. Soil inoculum

To obtain a bacterial inoculum for the microcosm studies the microcosm buffer was inoculated with a soil extract solution. The soil was a freshly collected ditch–bank spoils material that was collected from 45 cm below the bottom of an irrigation ditch. The extract solution was prepared by mixing 50 g wet weight of this soil with 50 mL of buffer and centrifuging at 500 $\times g$ for 5 min. One milliliter of the supernatant fluid was incorporated into 99 mL of microcosm buffer.

2.8. Analysis of CO_2 and N_2O

The accumulation of CO_2 and N_2O was used as a measure of microbial respiration and denitrification in the microcosms. Gas chromatography was used to analyze for CO_2 and N_2O in the atmosphere of the microcosms. A syringe sample from the atmosphere of the microcosm bottles was used to fill a 2.0 mL sampling loop connected to the inlet of the gas chromatograph. Peak separation was achieved using a 1 m Porapak Q column (Supelco), a 3 m Porapak Q column and a 1 m Shimalite column (Shimadzu) joined in series. All columns were 1/8-in. OD stainless steel, were operated at 90 °C, and were supplied with He at 30 mL min^{-1}. At 0.90 min after the injection the flow on the 1 m Porapak Q column was reversed to prevent slow eluting peaks (i.e. H_2O) from entering the 3 m Porapak Q column and the 3 m Porapak Q column was vented for the first 1.25 min of operation to prevent fast eluting peaks (i.e. N_2 and O_2) from reaching the Shimalite column. A thermal conductivity detector operated at 100 °C and at 100 mA was used to detect CO_2 and a ^{63}Ni electron capture detector operated at 200 °C and supplied with 5% methane in argon makeup gas at a flow of 30 mL min^{-1} was used to detect N_2O. The detectors were connected in series.

2.9. Statistical and curve-fit determinations

The Instat® v 3.05 computer program (GraphPad Software Inc.) was used for statistical comparisons. The program's two-tailed paired *t*-test and repeated measures analysis of variance were used to determine significance. Graphical curve-fit determinations were made using the Slide Write Plus for Windows v 5.01 graphics program (Advanced Graphics Software, Inc.).

3. Results

3.1. Effect of Tween on nitrate removal, microbial respiration and denitrification

If Tween 80 is to be used as a bioremediation aid then it is important that it does not have a negative impact on important microbial processes. The effect of Tween 80, at concentrations of 0%, 0.0125%, 0.025%, 0.05%, 0.1%, and 0.2% on nitrate uptake, nitrite formation, microbial respiration, or denitrification was evaluated in a

10-day microcosm study (Table 1). Nitrate disappeared rapidly in the microcosms and by the seventh day the nitrate present in all of the microcosms was essentially depleted. The disappearance of nitrate was not influenced significantly ($P=0.3008$) by the amount of Tween 80 present. Nitrite accumulated as a denitrification intermediate with the highest accumulation being observed on the fourth day of the incubation; the amount of accumulation was not influenced by the Tween 80 treatments ($P=0.5429$). CO_2 accumulations peaked on the fourth day of the study while N_2O accumulations were highest on the last day of the study. As with the changes that were observed with nitrate and nitrite neither headspace CO_2 accumulations (respiration) nor N_2O accumulations (denitrification) were significantly influenced by the Tween treatments, $P=0.8801$ and 0.4375 respectively. These results show that Tween 80 had no impact on overall nitrate uptake, nitrite formation, microbial respiration, or denitrification.

Table 1
Effect of different concentrations of Tween 80 on NO_3^- uptake, NO_2^- accumulation, headspace CO_2 and headspace N_2O

Incubation time	Tween 80 (%)	NO_3^-	NO_2^-	CO_2	N_2O
		(mM ± SEM)		(μM ± SEM)	
Day 0	0	11.1 ± 0.3	0 ± 0	0.5 ± 0.1	0 ± 0
	0.0125	10.8 ± 0.2	0 ± 0	0.3 ± 0	0 ± 0
	0.025	10.2 ± 0.3	0 ± 0	0.3 ± 0	0 ± 0
	0.05	11.2 ± 0.2	0 ± 0	0.3 ± 0	0 ± 0
	0.1	11.3 ± 0.0	0 ± 0	0.3 ± 0	0 ± 0
	0.2	11.8 ± 0.3	0 ± 0	0.4 ± 0	0 ± 0
Day 2	0	11.0 ± 0.2	0.8 ± 0.2	20 ± 2	0.5 ± 0.0
	0.0125	10.7 ± 0.3	0.8 ± 0.2	17 ± 2	0.4 ± 0.1
	0.025	11.1 ± 0.2	0.5 ± 0.1	14 ± 1	0.5 ± 0.1
	0.05	11.2 ± 0.1	0.6 ± 0.1	13 ± 2	0.4 ± 0.1
	0.1	11.4 ± 0.1	0.6 ± 0.1	12 ± 1	0.4 ± 0.1
	0.2	11.6 ± 0.2	0.5 ± 0.1	11 ± 0	0.3 ± 0.0
Day 4	0	2.8 ± 0.4	8.9 ± 0.3	79 ± 6	11 ± 2
	0.0125	3.1 ± 0.6	8.0 ± 0.5	77 ± 6	18 ± 5
	0.025	2.3 ± 0.4	8.8 ± 0.3	81 ± 3	19 ± 4
	0.05	2.4 ± 0.2	9.3 ± 0.3	82 ± 2	13 ± 2
	0.1	1.6 ± 0.2	10.0 ± 0.3	85 ± 3	17 ± 3
	0.2	2.4 ± 0.3	10.0 ± 0.4	81 ± 3	13 ± 3
Day 7	0	0.3 ± 0.2	5.3 ± 1.8	54 ± 17	44 ± 19
	0.0125	0 ± 0	4.0 ± 2.0	42 ± 14	78 ± 22
	0.025	0 ± 0	3.4 ± 1.6	59 ± 13	83 ± 17
	0.05	0 ± 0	4.9 ± 1.5	72 ± 12	64 ± 17
	0.1	0 ± 0	3.5 ± 1.8	57 ± 14	80 ± 21
	0.2	0 ± 0	7.3 ± 1.4	79 ± 11	52 ± 16
Day 10	0	0.2 ± 0.2	2.4 ± 1.8	55 ± 10	89 ± 20
	0.0125	0 ± 0	1.9 ± 1.5	51 ± 11	94 ± 15
	0.025	0 ± 0	0 ± 0	40 ± 2	110 ± 1
	0.05	0 ± 0	1.6 ± 1.3	41 ± 13	95 ± 14
	0.1	0 ± 0	1.4 ± 1.4	49 ± 9	97 ± 14
	0.2	0 ± 0	0.3 ± 0.2	31 ± 3	89 ± 7

3.2. Effect of Tween on emulsion stability

The emulsions were not stable at the lower Tween 80 concentrations. When the concentration of Tween in the aqueous phase was below 0.02% a visible layer of oil begin to form when beakers containing the emulsions were allowed to stand undisturbed. The oil layers begin to form within one-half hour and increased in size with time. Within 24 h almost all of the oil in the emulsions containing no Tween or 0.002% Tween 80 had separated from the aqueous phase and much of the oil (~80%) in the 0.01% and 0.02% Tween emulsions had separated. The emulsions that contained 0.05% or more Tween were more stable; these emulsions formed no visible oil layer on standing for 48 h (data not presented).

3.3. Column emulsifier studies

These studies evaluated the effect of Tween 80 on the initial distribution of an oil and water emulsion during the injection of the emulsion onto sand columns. The working hypothesis was that the addition of an emulsifier to the emulsion would increase the area covered by the oil during injection. Greater coverage would be desirable as it would decrease the number of injections needed to create an in situ preamble barrier. The study showed that when an emulsion containing 0.01% or less Tween 80 was injected onto a sand column the oil became trapped in the first 4 to 7 cm of the column. However, adding 0.02% to 0.1% Tween 80 to the emulsion before the injection increased the distribution of the oil in the column to 19 to 29 cm (Fig. 2). Thus, the results show that the addition of an emulsifier to the emulsion greatly increased the distribution of the oil in the sand during these injections.

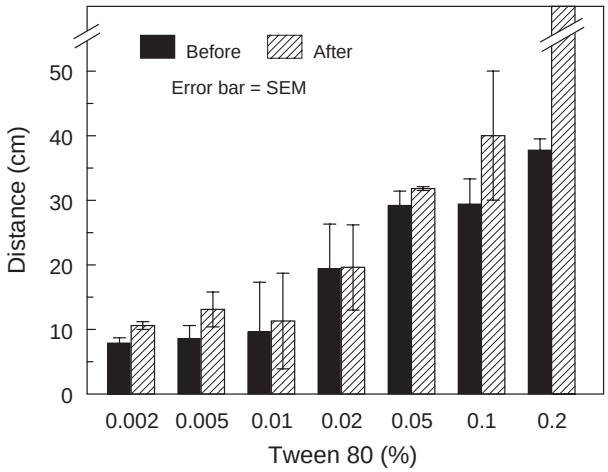

Fig. 2. Distribution of the oil emulsion in the sand columns immediately following the injection of the emulsion (before) and after 1.5 L (11 column void volumes) was pumped through the columns (after). At the 0.2% Tween 80 level much of the oil emulsion flowed out of the columns when the 1.5 L of water was pumped through these columns.

The addition of an emulsifier can also have undesirable effects on oil based stationary permeable barriers. There is a risk that the emulsifier used to aid in the distribution of the oil in the injection process may destabilize the barrier causing the vegetable oil to be carried away with the flowing groundwater, preventing the formation of a stationary barrier. The stability of the newly formed oil barriers was evaluated in short term, 1½-day flow studies conducted immediately after the oil emulsion was injected onto the columns. The results of these flow studies show that there was only a slight movement of the emulsion in the direction of flow when the amount of Tween 80 in the aqueous phase was 0.05% or less. However, at the 0.2% Tween 80 concentration much of the vegetable oil appeared to flush from the columns that received that amount of Tween (Fig. 2). The oil emulsion containing 0.02% and 0.05% Tween showed almost no movement in these short term flow studies. These results suggest that these amounts of Tween can be incorporated into the aqueous phase at the time of injection to improve the initial distribution of the barrier without destabilizing the barrier.

3.4. Long-term flow studies

Long-term, 60 day, flow experiments were conducted in the sand columns to determine if the stationary permeable barriers created by injecting an oil emulsion with 0.05% Tween would remain in place while water flowed through them for a long period of time. The results show that the stationary barriers were relatively stable under long-term flow conditions. Pumping ~60 L of water, 441 column pore volumes, resulted in only a slight shift in the distribution of the soybean oil on the columns. At the end of the study the treatment columns were found to contain as much emulsion, 103%, as was present in control columns that were not pumped for 60 days. There was a slight displacement of the oil emulsion downstream by the flowing water but there was no detectable loss of oil from the sand columns (Fig. 3). This demonstrates that permeable barriers formed by the injection of oil emulsions are likely to remain stable for extended periods.

3.5. Effect of 0.2% Tween 80 on barrier stability and oil movement

A series of flow experiments were conducted in the sand columns to determine if the stationary permeable barriers created by injecting an oil emulsion with 0.2% Tween would remain in place while water flowed through them. The results show that the barriers containing 0.2% Tween were not very stable (Fig. 4) when water was pumped through them whereas columns containing 0.05% Tween 80 were relatively stable. When 1.5 L of water, ~12 column pore volumes, was pumped through columns containing 0.2% Tween 80 a significant shift in the distribution of the soybean oil in the columns occurred and there was a large loss of oil from these columns. In the control columns, columns that received no Tween, the first 30 cm contained 4.8 ± 1.6 g (mean $\pm$ SEM) of oil per 100 g of sand. In columns that received 0.05% Tween the first 30 cm of the columns contained 4.9 ± 1.3 g of oil per 100 g of sand (Fig. 3), but in the columns that received 0.2% Tween only 1.3 ± 0.3 g of oil per 100 g of sand was present in the first 30 cm (Fig. 4), a loss of almost 73%. And, at the end of the study the columns that received the 0.2% Tween emulsion were found to contain only 7.0 ± 1.1 (mean $\pm$ SEM) g of oil, 56% of the 12.5 g

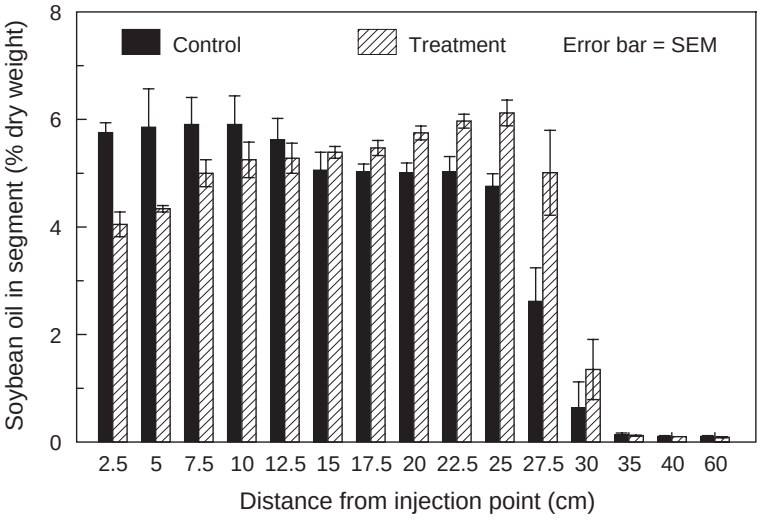

Fig. 3. Distribution of soybean oil along the length of the sand columns immediately after an oil and water emulsion containing 0.05% Tween 80 was injected (control columns) and distribution of the emulsion after water was pumped through the columns for 60 days (treatment columns). Each point is the average of 3 measurements ± SEM.

of oil that was originally injected onto the columns. Interestingly, pumping additional amounts of water through these columns did not result in additional losses of oil from the columns that received 0.2% Tween (Table 2).

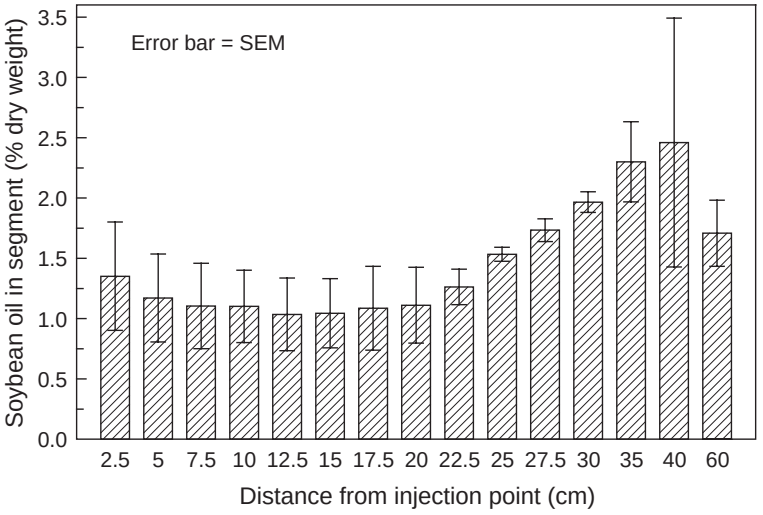

Fig. 4. Distribution of soybean oil along the length of the sand columns after an oil and water emulsion containing 0.2% Tween 80 was injected and after water was pumped through the columns for 60 days. Each point is the average of 2 measurements ± SEM.

Table 2
Effect of pumping water through sand columns containing 0.2% Tween 80 on the retention of soybean oil in the sand

Volume of water pumped through the columns (L)	Amount of oil remaining in the first 30 cm of sand (g)
1.5	3.6
6	2.3
12	2.9
18	3.9
24	2.6

3.6. Sand tank injections

Sand tank studies were conducted to determine the effect of high-pressure injection on the distribution of the vegetable oil in sand. In an initial study, sand tank injections of the oil and water emulsion were conducted at 70, 1400, and 18,000 KPa and the size and shape of the patterns, created by the dyed oil, noted. The 70 KPa injection (50 L was injected at a rate of 0.07 L min^{-1}) produced a circular pattern with a radius of about 22 cm with the injection point near the center of the circle. The 30-s 1400 KPa injection (9.9 L at 0.33 L s^{-1}) produced an oblong spherical shaped pattern with a length of 29 cm and a width of 22 cm. With this injection the point of injection was located about 6 cm from the edge of the pattern rather than in the center of the pattern. The 30-s 18,000 KPa injection (6.6 L at 0.22 L s^{-1}) produced a 42 cm long and 17 cm wide peanut shaped dye pattern with the injection point near one end of the pattern (Fig. 5). As was expected, the longest injection pattern was obtained with the 18,000 KPa injection and all additional studies were conducted at this pressure.

The dye served as a very good indicator for the presence or absence of oil in the sand and the dye pattern served as a good indicator showing what happened to the oil following an injection. Unstained sand, sand adjacent to but outside of the dye pattern was routinely analyzed for oil, using combustion analysis, and was always found to contain no oil.

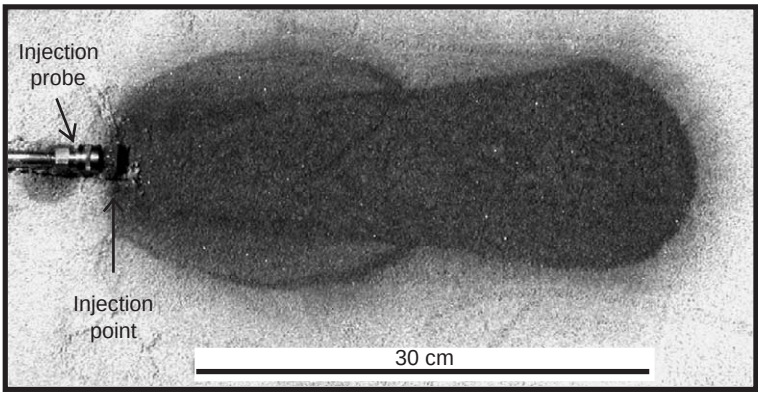

Fig. 5. Pattern created by the injection of a soybean oil and water emulsion into sand at 18,000 KPa for 30 s and at a flow of 0.22 L s^{-1}. A tracer dye was incorporated into the emulsion. The sand above the injection point was removed to reveal the distribution pattern.

Conversely, sand stained by the dye was always found to contain oil. Data on the distribution of oil in the dye patterns is presented later.

Following the initial study a series of 18,000 KPa (0.22 L s^{-1}) injections were made. These injections were made for 15, 30, 60 and 120 s. The longest patterns were produced by the longest injection times, 60 and 120 s. With the 60 s injection the oil emulsion penetrated the sand 46 ± 1.8 cm from the tip of the injection probe and formed a pattern 19.9 ± 2.3 cm wide with an area of 924 ± 22 cm^2 (mean $\pm$ SEM). Increasing the injection time to 120 s produced an injection pattern 49 ± 0.4 cm long, 24.6 ± 1.4 cm wide, with an area of 1034 ± 41 cm^2, a very small increase in the length of the injection pattern over that seen with the 60 s injections. Injections of greater duration were not attempted, as it was obvious from the data (Fig. 6, open symbols) and the curve-fit equations that increased injection times would yield only slight increases in the length, width, or area of the injection patterns.

The presence of 0.05% Tween 80 did not significantly ($P=0.384$) influence the length of the injection patterns (Fig. 6A) but did significantly ($P=0.0298$) increase the width of the injection pattern (Fig. 6B). The total area covered by the injection pattern (Fig. 6C) was also not significantly ($P=0.0989$) increased by the presence of Tween. Tween 80 concentrations of 0.02% and 0.1% in the aqueous phase also had no influence on the length of the injection pattern but gave an increase in the width of the pattern (data not presented). Adding 0.2% Tween 80 to the aqueous portion of the emulsion resulted in a very large injection pattern that was distributed throughout the sand tank (data not presented). These results suggest that 0.05% Tween 80 could be used to increase the width of an injection pattern but that the Tween at that concentration will not increase the length of the injection. Tween at a 0.2% concentration greatly increased the length of the injection

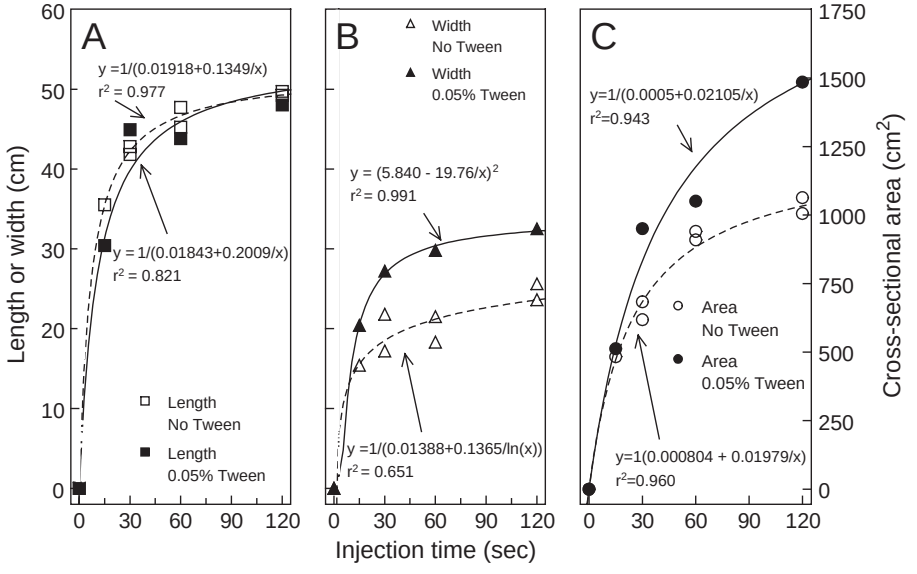

Fig. 6. Effect of time and detergent additions (Tween 80) on reach (A) and width (B) and area (C) of injection patterns. Injections were at 18,000 KPa. Each point represents a single data point.

pattern but may not have produced a stable oil containing barrier. These results are in agreement with the column studies that were conducted earlier.

The distribution of the oil within the injection pattern can influence the functioning of an oil barrier. If the concentration of oil within the barrier is too low then the functional life of the barrier may be less than desired. However, the greater the concentration of oil in the barrier the more that the oil will interfere with the movement of groundwater through the barrier (Hunter, 2001b). The amount of oil contained in the sand inside of the injection patterns was determined for a series of 18,000 KPa injections (Fig. 7). When injected for 120 s, the injected area contained 6.6 ± 0.6 (mean $\pm$ SEM) g oil per 100 g dry sand between the injection point and the 48 cm sample. When the injection time was decreased to 60 s, the amount of oil present in the sand was 6.9 ± 0.6 g oil per 100 g dry sand between the injection point and the 42 cm sample. With the 30 s injection the amount of oil present was 7.4 ± 0.2 g oil per 100 g dry sand between the injection point and the 36 cm sample. And, with the 15 s injection the amount of oil present was 10.6 ± 1.2 g oil per 100

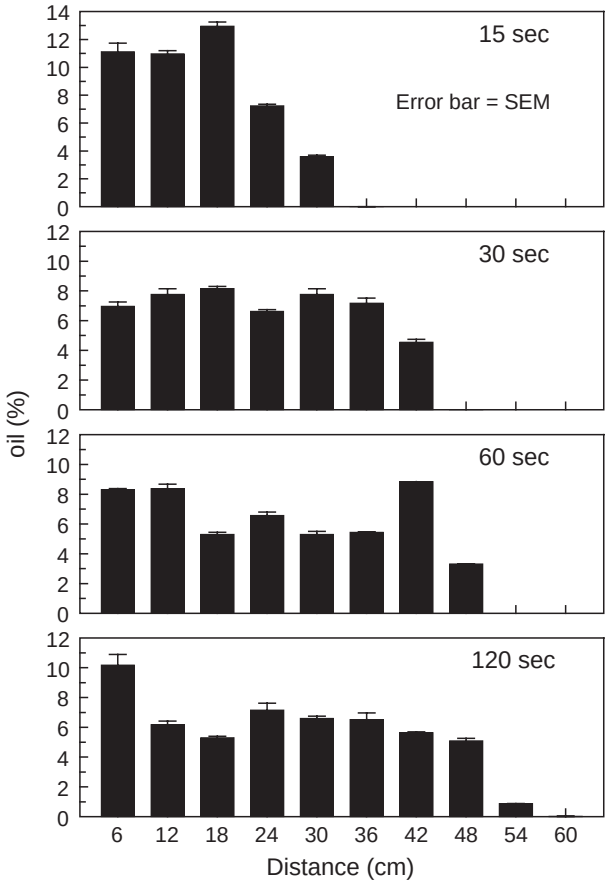

Fig. 7. Distribution of oil injected into the sand tank for 15, 30, 60 and 120 s. Injections were at 18,000 KPa with 0.05% Tween 80. Error bar = SEM of the combustion analysis, $n = 3$.

g dry sand between the injection point and the 24 cm sample. There was no tendency for large amounts of oil to accumulate in the sand near the injection point; rather, the distribution of the oil along the length of the injection patterns was relatively uniform for each of the injections.

4. Discussion

Injection may be used for the construction of permeable in situ barriers. The present study suggests that a multi-ported injection probe could be used to create stationary in situ permeable barriers that contain innocuous vegetable oil as the substrate for remediation. This study modeled the injection of an oil emulsion from a single injection point and demonstrated that an oil emulsion can be injected from an injection point into fine-grained sand up to a distance of approximately 50 cm. Using a multi-ported injection probe the oil could be distributed across an area approximately 1 m in diameter. In addition, higher mass flows might result in a larger distribution of the oil; in this study flow was limited to 0.22 L s^{-1} at the 18,000 KPa pressure. Pressures of 18,000 KPa were used for most injections during this study but lower pressure injections, 1400 KPa, produced oil dispersions that were almost as large as the 18,000 KPa injections. The data predicts that little would be gained from higher-pressure injections as long as the same physical constraints govern the distribution of the oil. During the injection, large amounts of oil did not accumulate at the injection point; rather, the distribution of oil within the sand was relatively uniform throughout the oil dispersion pattern.

The amount of oil provided by the injection was sufficient to treat a considerable amount of contaminated water. When an oil emulsion containing 25% soybean oil was used the amount of oil present in the sand pore space was 6.6 ± 1.6 (mean $\pm$ SD) g oil per 100 g dry sand (120 s injection). This amount of substrate would be sufficient to remediate a considerable amount of contaminated groundwater under most in situ flow conditions. Past work with laboratory columns has shown that 1-g of soybean oil will remove 0.224 g of nitrate-N from oxygenated flowing groundwater (Hunter et al., 1997). The longevity of oil based remediation barriers has not been determined but studies with sawdust and cellulose suggest the in situ barriers may have a functional life of many years. Robertson and Cherry (1995) field-tested a sawdust based in situ permeable barrier used for the removal of nitrate. This barrier contained 2% carbon and they estimated that the barrier should have a life of over 20 years. Blowes et al. (1994) estimated that a cellulose based barrier containing 5% carbon as cellulose might last for decades without the need for additional substrate.

In the sand tank studies, the addition of Tween 80 to the aqueous phase of the emulsion had no significant impact on the length of the 18,000 KPa injection patterns when the amount was less than 0.1%. At concentrations below 0.1%, Tween 80 did increase the width of the injection patterns and may be of some use as a distribution aid but the contribution is relatively minor.

This paper has concentrated on the creation of stable in situ barriers; barriers that remain in place after injection and that do not tend to move with the flow of the groundwater. However, some movement of the oil may be desirable, as a remediation

strategy, under many conditions. Movement would cause the oil to flow away from the injection point and could greatly increase the distribution of the oil substrate within the subsurface area being treated and would greatly decrease the number of injection points needed, decreasing the cost of the remediation project. This would be especially important when the aquifer being treated is deep and the cost of each injection is high. Under such conditions, the addition of enough emulsifier to cause the oil to move could be beneficial. The addition of 0.2% Tween 80 to the aqueous phase of the emulsion was a sufficient amount of emulsifier to greatly increase the mobility of the oil. However, enough oil would need to remain trapped in the pore space to provide an effective barrier. Additional study is needed to determine the effect of higher concentrations of Tween on the effectiveness and efficiency of oil based barriers.

The influence that injected oil may have on the hydraulic conductivity of the aquifer is an issue of concern. Schipper et al. (2004) investigated the use of a sawdust based barrier to remove nitrate from flowing groundwater and found that the barrier interfered with the hydraulic flow of the groundwater causing most of the groundwater to flow around rather than through the barrier. This greatly reduced the effectiveness of the barrier. In contrast, Robertson and Cherry (1995) and Robertson and Anderson (1999) were successful in removing nitrate from contaminated groundwater with a denitrification barrier and Henry et al. (2003) were successful at removing chlorinated ethenes with a 139-m long, by 7.3-m deep, by 0.46-m wide mulch barrier. That these barriers were successful at removing the targeted contaminants suggests that a large percentage of the groundwater flow must have passed through the respective barriers. Little is known about the effect that injected soybean oil emulsions might have on hydraulic conductivity and more study is clearly needed in this area. Wiedemeier et al. (2001) have injected vegetable oil into aquifers contaminated with trichloroethylene and have detected trichloroethylene degradation products suggesting that the remediation procedure was successful. They did not emulsify the injected oil; rather oil was injected directly into the aquifer at multiple points in order to produce pools of oil that slowly release organic carbon into the aquifer. This released carbon is thought to have stimulated the biological reduction of the trichloroethylene.

Other problems may also occur with the use of remediation barriers, be they based on vegetable oil or on other electron donors, and it is important that care be used in the implementation of such barriers. For example, if excessive amounts of electron donor are used and the electron acceptor becomes depleted then problems with methane formation may occur. Conversely, if the supply of electron donor limits the reduction of the electron acceptor then undesirable degradation products may accumulate. The reduction reaction may also fail to go to completion if an important nutrient is limiting. In laboratory studies nitrite has been shown to accumulate in large amounts in denitrification barriers when the availability of phosphate limited the denitrification process (Hunter, 2003). Also, the presence of heavy metals and pesticides might interfere with the proper functioning of denitrification barriers causing nitrite to accumulate (Mitsui et al., 1964; Bollag and Henneringer, 1976; Bollag and Barabasz, 1979; Bollag and Kurek, 1980; Hunter and Kuykendall, 2005) and may also interfere with the functioning of barriers used for other types of remediation. These concerns do not mean that remediation barriers should not be used but it does mean that factors that influence their proper functioning should be understood and considered when they are used.

Innocuous vegetable oils can be used as a substrate to stimulate the bioremediation of a number of groundwater contaminants. Past studies have used vegetable oil to remove nitrate, perchlorate, chlorate, and chlorinated solvents from water (Hunter and Follett, 1995; Hunter et al., 1997; Wiedemeier et al., 2001; Lee et al., 2001; Hunter, 2002; Waddill et al., 2002) and many other contaminants may also be removed by such barriers. Trenches backfilled with oil-coated sand to create oil based permeable barriers could be used to establish the barriers (Hunter, 2001b) or the permeable barriers may also be created by injecting an oil emulsion into a contaminated aquifer. It may not be necessary for the injection to create a continuous barrier of oil containing material across the aquifer. The field studies by Wiedemeier et al. (2001), discussed above, suggest that discontinuous barriers may also stimulate significant remediation.

5. Conclusions

Bioreactor studies showed that low concentrations of Tween 80 had no measurable impact on microbial denitrification or respiration and thus should be safe to use as a bioremediation aid. Column studies demonstrated that Tween 80 can be used to increase the distribution of oil emulsions injected at low-pressure. Tween at 0.02% to 0.05% resulted in a controlled increase in the distribution of the oil in the sand columns and in the formation of column based barriers that were stable over the 60 day study. The addition of larger amounts of Tween, 0.2%, to the water used in the oil emulsion resulted in much greater oil mobility and in the loss of much of the oil from the sand columns. This greatly increased mobility may be of value as a distribution aid in some remediation situations but was not studied in detail here.

The sand tank studies show that pressure can be used to distribute emulsified oil. The longest injection patterns, ~0.5 m, were achieved at the highest pressure, 18,000 KPa. Adding small amounts of Tween increased the width but did not increase the length of these high-pressure injection patterns. Pressure injection at multiple points used with a multi-ported injection probe might be used to create in situ permeable barriers approximately 1 m wide in water-saturated fine-grained sand.

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